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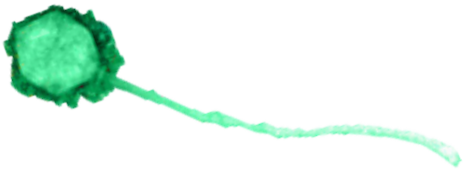

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Bacteriophages, viruses that infect bacteria, have numerous applications in the medical, agricultural, and research fields, especially as an alternative to antibiotics in the age of antibiotic resistance. Phages are able to lyse, or break apart, bacterial cells with fewer side effects, more specificity, and less likelihood of resistance than antibiotics. The acceptance of phages in medicine and agriculture around the world today is not universal, and the United States Food and Drug Administration (FDA) has been slow to recognize phage therapy as a legitimate treatment. However, the successful use of phages in the past, as well as promising trial results in fields ranging from chronic disease treatment to food preservation, present opportunities for consideration of phage-based applications in the future. The goal of this paper is to provide an overview of the history, uses, and regulation of phage therapy.

Introduction

If you were to walk out into your backyard today, you probably wouldn't notice the thousands of tiny microorganisms living there right underneath your feet. Many of these microorganisms would be bacteria, but at least ten times as many would be their viral predators (Hattful, 2008). These bacteria-infecting viruses, called bacteriophages or "bacterium-eaters," were discovered in 1910 by Felix d'Herelle, a French-Canadian microbiologist (Golkar, Bagasra, & Pace, 2014). One example of a bacteriophage is shown in Figure 1. They can be found almost anywhere on Earth and are a topic of increasing study, especially due to the dramatic rise of antibiotic resistance. Bacteriophage research is conducted worldwide in an effort to combat this resistance and address a variety of health issues, ranging from chronic diseases to safety in the food industry.

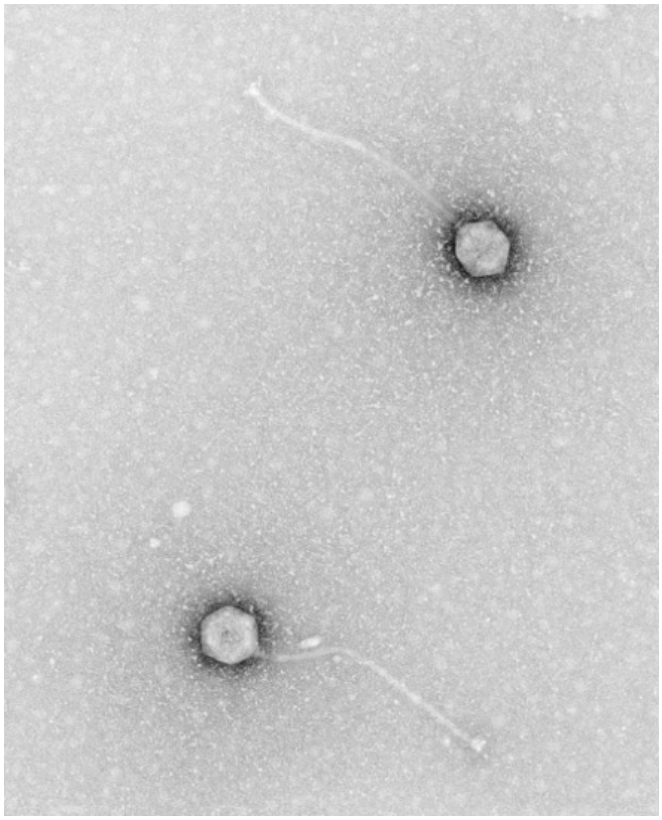

Figure 1. An electron micrograph image of a siphovirus isolated by Isabel Jimenez-Bush from a soil sample in a JMU Viral Discovery class. Note the icosahedral head along with the long flexible tail.

History of Phage Therapy

The use of bacteriophages to treat pathological bacterial infections, called phage therapy, began in France in 1919. By the 1930s, phage therapy was in use in Europe and the United States (Highfield, 2014). After the introduction of antibiotics, however, phage therapy became virtually obsolete everywhere except Eastern Europe and the Soviet Union. The decline in phage therapy was the result of multiple factors, including the cessation of research communication between Eastern Europe and the Western world during World War II and the counterintuitive nature of using viruses to treat diseases (Summers, 2012). In addition, the difficulty in regulating a virus as a therapeutic agent is still a major barrier.

During World War II, the Allied and Axis powers differed in regard to method of treatment for bacterial infections. While German scientists studied phages, the Allies were heavily dependent on antimicrobial drugs like penicillin and amoxicillin (Summers, 2012). The efficacy and speed of these pharmaceuticals were a major breakthrough in the medical industry, surpassing the still-developing phage therapy. Antimicrobial drugs were produced in great quantities, rendering phage therapy virtually obsolete. Recently, the emergence of bacterial resistance to antibiotics has caused researchers to seek alternative methods of treatment (Yosef, Kiro, Molshanski-Mor, Edgar, & Qimron, 2014).

Although ignored for several decades in the West, the decline of phage therapy was neither uniform nor complete, as evidenced by its continued use in the Republic of Georgia by the Tbilisi Institute (Kutateladze & Adamia, 2008). No substantial research or well-documented practice of phage therapy arose for the next 40 years; however, in the last few decades phage therapy research has reemerged in the Western world to combat the urgent problem of antibiotic resistance.

Current Uses and Future Applications of Phages

While many phage treatments are not yet supported by the United States' Food and Drug Administration (FDA), research has shown phages to be beneficial in areas like food preservation, agriculture, livestock health, and the treatment of human infections. Several uses that will be discussed include overcoming antibiotic resistance, sterilizing medical equipment, and treating agricultural products.

A promising technique for overcoming antibiotic resistance involves using specific proteins from phages that lyse bacteria, and can be isolated and used to treat bacterial infections. Phage therapy significantly increases the ability to combat antibiotic resistance in bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* with 90% efficiency (Pirisi, 2000). These bacteria are known to cause a variety of health problems like staph infections, pneumonia, bronchitis, meningitis, and gastroenteritis, respectively. Additionally, phages are effective against ten of the most antibiotic-resistant strains of *E. coli* (Rahmani, Zarrini, Sheikhzadeh, & Aghamohammadzadeh, 2014). Table 1 shows several comparisons between bacteriophages and antibiotics and offers a condensed view of phage therapy viability. The

Table 1. Comparing and Contrasting Bacteriophages and Antibiotics

	Side Effects	Targeting Ability	Site Specificity	Resistance	Treatment Development
Bacteriophages	No serious known side effects	Infect targeted pathogenic bacteria	Congregate at infection site	Rarely encounter phage-resistant bacteria which are typically susceptible to other phages	Can easily select specific phages to develop new treatments quickly; new phage treatments are easy to develop due to high specificity of phages
Antibiotics	Can cause side effects such as allergies, intestinal disorders, and secondary infections	Target both pathogenic microorganisms and normal microflora, which affects microbial balance in patients	Do not concentrate at the infection site and are eliminated from the body over time	Antibiotic-resistant bacteria are common and develop from non-selected bacteria during antibiotic treatment	New antibiotics are developed slowly

Note. Chart adapted from "Bacteriophage Therapy," by A. Sulakvelidze, Z. Alavidze, and J. G. Morris, 2001, *Antimicrobial Agents and Chemotherapy, 45*(3), 649–659.

use of phages as antibacterial agents in the past century in other places than the United States has had a lasting impact on the treatment of bacterial infections (Abedon, Kuhl, Blasdel, & Kutter, 2011).

Regulating Applications of Phage Therapy

The policy decisions regarding characterization of phages as medicinal products are controversial due to the varying definitions of bacteriophages and efficacy of phage therapy. In many countries, phage therapy is placed into one or more of three categories: a general medicinal product, a biological medicinal product (such as a vaccine), or an advanced medicinal product (such as gene therapy). In Europe, phage therapy is already implemented and is considered a biological and medicinal product suitable for human use (Verbeken et al., 2014). The European Regulatory Framework dictates that phage therapy should be delivered through a mixture of bacteria-specific phages also known as "phage cocktails." For non-life-threatening infections, these cocktails are sold over the counter. Phage cocktails that target potentially life-threatening bacterial infections, however, are only administered in a hospital setting (Verbeken et al., 2014).

Phage Cocktail Therapy has yet to be fully supported by the FDA for direct treatment of human disease because each phage must be approved individually (Keen, 2012). An example of the FDA's hesitation toward this type of therapy is the production of Staphylococcal Phage Lysate (SPL) in 1959. SPL can be used to treat wounds infected with *Staphylococcus aureus* and after safety trials were conducted, SPL was licensed for administration in human treatment. However, the production of SPL for human treatment was suspended in the 1990s due to regulatory pressure from government agencies. Currently, SPL is approved and utilized only for veterinary applications (Golkar et al., 2014).

In regard to the food industry, in 2006 the FDA approved phage food additives to combat contamination, particularly for anti-Listeria treatment ("Food Safety," 2015). Phages can be used to treat Listeria in ready-to-eat meat, and bacterial damage of tomatoes and peppers, and treatments are recognized as compatible with organic food production. Additionally, the FDA has approved phage use in treatment of bacterial pathogens such as *E. coli*, *Salmonella*, and *Campylobacter* (Golkar et al., 2014).

Phage Therapy and *Campylobacter jejuni*.

The use of phages in the food industry includes the treatment of the *Campylobacter jejuni* bacterium. The *Campylobacter jejuni* bacterium is the most common cause of gastroenteritis in humans. Commonly found in the feces and intestinal tracts of poultry, the *C. jejuni* bacterium causes contamination of poultry meat. One method of preventing infection involves dosing live poultry with bacteriophage cocktails to lessen the amounts of *C. jejuni* in their intestinal tracts (Hammerl et al., 2014).

Hammerl et al. (2014), studied the effects of the introduction of the Myoviridae bacteriophage family on the *C. jejuni* bacterium found in broiler chickens. In this study, some chickens were treated with single phages and others with groups of phages (Hammerl et al., 2014). The phages were

categorized into three groups based on genome size and morphology. Phages in group II were able to infect more strains of bacteria than group III, though some of the phages in group III had a higher lytic activity, meaning they were more effective at killing the bacteria of the strains that they were able to infect. After comparing the ability of these phage groups to reduce *C. jejuni* numbers, the researchers found that the chickens treated with a combination of phages from groups II and III had the greatest reduction of *C. jejuni* in their intestinal tracts (Hammerl et al., 2014). The results of this study could positively impact the future of food safety.

Phage Therapy and *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa is an opportunistic, multidrug-resistant bacterium commonly found among the human microbial flora of the skin. However, if this seemingly harmless bacterium is introduced into the bloodstream, it can cause serious complications, including sepsis, an often fatal bacterial infection of the blood (Vieira et al., 2012). Vieira et al. (2012) demonstrated in their study that immediately after phages were applied to infected human skin the amount of resistant bacteria significantly decreased from a control of 10^6 CFU (colony forming units) per square centimeter to 10^2 CFU per square centimeter. This nearly ten billion CFU per square centimeter drop supports the effectiveness of phage therapy. In addition, the experiment exhibited that only one application of phages was necessary as subsequent applications did not lower the bacterial concentration. This proves promising for treating multidrug-resistant bacterial infections using phage therapy (Vieira et al., 2012).

Potential Use of Phages in Treatment of Crohn's Disease

Another use of phage therapy in medical research can be seen through the treatment of Crohn's disease (CD). CD is commonly called regional enteritis, or inflammatory bowel disease, and its cause is unknown. Josef Wagner, researcher at Murdoch Children's Research Institute, has investigated the differences in the bacteriophages present within the gut and small intestine of pediatric patients that have CD (Wagner et al., 2013). The researchers analyzed the genome of the phages to determine if these phages were unique to the intestines. Ultimately, they found that CD patients exhibited a higher amount of unique bacteriophages compared to their control counterparts. Overall, the large abundance of phage composition within CD ileum tissue and CD gut wash samples suggests a role of phages in CD development. Researchers assumed that the increase in the amount of phages indicated that the bacterial count was higher. Therefore, the presence of specific phages may coincide with specific bacterial counterparts (Wagner et al., 2013). Because phages are host-specific, the identification of disease-causing phages could allow for development of new and effective treatments.

Phage Application in Cleaning Surfaces

Another environment prone to resistant bacteria is hospital equipment. Bacterial colonies growing on the surfaces of hospital equipment often come into contact with antibacterial cleaning supplies. This common interaction allows the bacteria to develop resistance to these cleaners. Research is being conducted to devise a technique to clean hospital surfaces while treating resistant bacteria using bacteriophages (Viertel, Ritter, & Horz, 2014). These cleaners prove less of a hurdle for regulation due to the fact that they would not be used directly on humans.

Susceptibility in Antibiotic-Resistant Bacteria

Another technique to combat antibiotic resistance is to use phages that insert their genome into the host bacterium to transfer susceptibility genes back into the bacterial genome (Viertel et al., 2014). This allows bacteria that were once antibiotic resistant to regain susceptibility to antibiotics. This occurs because the genes that are transferred into the bacterium increase the permeability of its cell membrane and allow medications to pass through it more freely. Therefore, doctors will be able to use antibiotics that they are familiar with, an approach that is more likely to be accepted by the FDA (Viertel et al., 2014).

The identification of disease-causing phages could allow for development of new and effective treatments.

Endolysins

Endolysins are phage-produced enzymes that rupture a bacterial host cell. These endolysins are specific proteins with known structures that can be defined and therefore regulated. The controversies surrounding use of whole phages or mixtures of phages, including their chemical and biological classification, may ultimately inhibit the approval of phage therapy in the U.S. However, endolysins may provide a solution. As endolysins are static chemicals similar to antibiotics, they may prove more successful in regulation and approval by the FDA.

In addition, endolysins are only required in small doses (Ghannad & Mohammadi, 2012). Researchers at Seoul National University in South Korea studied the use of a phage called PCB1 against *Bacillus cereus*, a bacterium known to cause food poisoning in humans. PCB1 itself was only able to successfully infect 1 out of 22 *B. cereus* strains; however, the purified endolysins produced by the phage were able to successfully lyse all strains of *Bacillus* bacteria (Kong & Ryu, 2015). The lytic activity of the endolysin suggests that these chemicals not only appear to be more favorable due to their static nature, but also may have broader and more successful applications against bacteria than phages themselves

Combination Therapy

The most successful phage therapy is the combined usage of bacteriophages and antibiotics. By using this technique, treatment has shown a promising increase in the reduction of bacterial infection and reducing the amount of bacteria evolving to develop antibiotic resistance. In the future, using both bacteriophages and antibiotics to fight infections may appease drug companies that seek the opportunity to continue producing their own medications, while increasing the effectiveness of phage therapy treatment (Viertel et al., 2014).

Conclusion

Phage therapy presents numerous advantages and options for medical research and scientific application. It is not currently in use in the United States due to a lack of regulatory ability and a large-scale control study, as well as the controversies surrounding the use of phages. New routes of regulation and classification will have to be established for phages to enter mainstream use in the U.S. Phages are being studied with broader host ranges, and being tested on their ability to kill pathogenic bacteria without harming the patients or destroying the body's natural flora. Other areas of phage research include sterilization techniques for hospital equipment, endolysin usage that may surpass phage usage alone, and combination of phage and antibiotic therapies that could potentially result in approval for future use of phages. Phage research has also shown promising results in reintroducing antibiotic susceptibility into antibiotic resistant bacteria, which has become an increasingly prevalent issue in the modern medical world. In addition to combatting antibiotic resistance, phage therapy could eventually be used as a more effective alternative for bacterial infections. For these reasons, phages are a promising medical and industrial tool worldwide in the future.

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